SECTION HEAD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 22 1986

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

QC030001

MEMORANDUM:

Subject: Pathology Report on 2,4-D from Dr. A. Koestner, Michigan State University

To: Lois Rossi
Special Review Branch
Registration Division (TS 767C)

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED
M. van Gemert 7.22.86

Thru: Theodore Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division
Theodore M. Farber 7/22/86

Compound: 2,4 Dichlorophenoxyacetic acid

Tox.Chem No.: 315

Registrant: Industry Task Force on 2,4-D Research Data

Action Requested: Review Dr. Adalbert Koestner's pathology report on 2,4-D.

Conclusions: Dr. Koestner has concluded that the incidence of astrocytomas in the brains of rats treated with 2,4-D is not a treatment-related phenomen. His arguments for this conclusion are summarized below. These comments will be taken into consideration along with our own independent evaluation of the slides and peer review before reaching a final conclusion concerning the possible carcinogenicity of 2,4-D.

Discussion: Dr. Koestner's conclusions concerning the tumor incidence from re-evaluating the tumor sections of the animals diagnosed as having astrocytomas are summarized in the table below:

TABLE I

TUMOR INCIDENCE

	Male	Female
Controls	1/60	0/60
1 mg/kg	0/60	1/60
5 mg/kg	0/60	2/60
15 mg/kg	2/58	1/60
45 mg/kg	5/60	1/60

Dr. Koestner does not feel that the tumor seen in the high dose male (case # 23473) found after additional brain tissue was imbedded and sectioned, is actually neoplastic. His diagnosis is that it consists of a mixed glial and mesenchymal cell population, and he therefore did not include it in table 1. In his experience, "most early astrocytomas are remarkably monomorphic and elicit no tissue reaction. In the animal # 23473, the tumor consists of pleomorphic perivascular and dispersed cell populations including granulocytes and lymphocytes in addition to glial tissue. Special stains in this case reveal reticulin and collagen formation which is a function of specific mesenchymal cells but not of astrocytoma cells." The tumors which were diagnosed as astrocytomas were all of glial origin, generally well differentiated with little tissue reaction by surrounding brain tissue. In some animals there were areas of necrosis with some subsequent repair responses evident. These tumors Koestner considered to be identical to glial tumors routinely found in aged rats.

BIOLOGICAL CRITERIA FOR EVALUATION OF NEUROCARCINOGENS

Depending on the experimental design, carcinogenic potential of the compound and availability of tissue samples at various stages during the experiment, some or all of the following criteria may apply to or are testable in any single case. Some according to Koestner, will always be present and he states will permit a distinction to be made between experimentally induced and naturally occurring brain tumors.

1. Increased incidence beyond expected control levels,
2. Shift of tumor appearance to a younger age (decreased survival time),
3. Demonstration of dose-effect relationship,
4. Higher tumor incidence after transplacental exposure,
5. Trend toward anaplasia,
6. Presence of preneoplastic lesions,
7. Multiplicity of tumors in individual animals,
8. Tumor occurrence in both sexes,
9. Tumor occurrence also in peripheral nervous system,
10. Tumor induction outside the nervous system.

11. Genotoxicity. mutagenicity. chromosomal aberrations.

His arguments against any biological evidence for carcinogenesis are listed below in order.

1. Increased incidence beyond expected control levels:

Dr. Koestner presented a table of incidences of gliomas in control male Sprague-Dawley rats found by commercial laboratories with an incidence range of 0-10%.

TABLE II

VARIABILITY IN BRAIN GLIOMA INCIDENCE IN CONTROL MALE SPRAGUE-DAWLEY RATS 1 YR. AND OLDER (SELECTED FROM SWENBERG, J.A. 1986)

NUMBER	COLOR	LABORATORY	CONTROL 1(%)	CONTROL 2(%)	CONTROL 3(%)
1-5	Diff. Colors	IRDC	0/292 (0)	2/287 (0.7)*	2/137 (1.4)
6	Red No. 33	IRDC	3/57 (5.2)	0/59 (0)	2/58 (3.4)
7	Green No. 3	Biodynamics	0/52 (0)	5/55 (9)**	--
8	Blue No. 2	Biodynamics	0/59 (0)***	2/59 (3.4)	--
9-13	Diff. Colors	Biodynamics	2/290 (0.7)	2/289 (0.7)	4/231 (1.7)
14	Red No. 9	Litton	4/58 (6.9)****	6/60 (10)	2/57 (3.5)
15	Red No. 27	Litton	2/54 (3.7)	0/55 (0)	--
16	Red No. 36	Litton	2/57 (3.5)	1/59 (1.7)	0/53 (0)
17	Red No. 30	Hazleton	3/59 (5.1)	1/55 (1.8)	--

* One of the rats died on day 350 with a glioma
** Additional sections resulted in 6/55 (10.9%)
*** Additional sections resulted in 2/59 (3.4)%
**** One glioma diagnosed at 12 mos. interim sacrifice

In the F344 rat strain NCI carcinogenicity studies show an incidence range of gliomas in 4700 male and female rats of 0-3.3%. Dr. Koestner also quotes a reported incidence range of 2.8% in males and 1.6% in female F344 rats from a paper by Soleveld, et al. Koestner emphasized that these incidences were reported for only 3 sections per brain being examined. Since these gliomas are "microtumors", the harder one looks for them, the more likely they are to be found. In the 2,4-D study 7 blocks per brain were examined as compared to the standard 3. So these reported incidences may be under-estimating the actual historical control incidences.

2. Shift of tumor appearance to a younger age:

Dr. Koestner states that usually with space-occupying brain

tumors in the restricting cranial cavity, there is a shortening of survival. However, this shortening of survival is not seen in 2,4-D exposed rats, but can be demonstrated in a number of neuro-oncogenic substances.

3. Demonstration of a dose-effect relationship:

Other neuro-oncogens such as ethyl nitrosourea and methyl methanesulfonate show a dose-response relationship. However, no such dose-effect relationship exists for 2,4-D. There is an unequal clustering of tumors in the high dose group only, according to Dr. Koestner.

4. High tumor incidence after transplacental exposure:

This criterion cannot be evaluated because it hasn't been tested.

5. Trend toward anaplasia:

The tumor spectrum, according to Dr. Koestner, in the 2,4-D study was comparable to that found in surveys of the spontaneous brain tumors in rats. He claims the tumors are primarily of mature and differentiated astrocytic cell populations. However, in other neuro-oncogens, such as MNU, 53% of the brain tumors produced were either unspecified gliomas (14%), anaplastic gliomas (14%) or gliosarcomas. In addition methyl methanesulfonate, a weak carcinogen, produced primarily malignancies. Five of the 7 tumor-bearing animals had malignant neurogenic neoplasms (most rats had several tumors).

6. Presence of preneoplastic lesions:

Neurocarcinogens such as ENU can elicit early preneoplastic glial proliferations with glial tumors appearing much later. Koestner believes that these early neoplastic glial proliferations are good indicators of chemical tumor induction as opposed to spontaneous neoplasms in older rats. None of these preneoplastic lesions were seen in the rats given 2,4-D.

7. Multiplicity of tumors in individual animals:

Koestner states that a multiplicity of neurogenic tumors is the rule rather than the exception for compounds such as ENU or MNU. However, no rat in the 2,4-D study had more than one neurogenic tumor.

8. Tumor occurrence in both sexes:

In the 2,4-D study Koestner claims that the incidence in male

rats is slightly higher than in female rats (2.6 to 1.6%), as is the case under natural conditions. Females in the 2,4-D study didn't show any increased tumor incidence.

9. Tumor occurrence in both the central and peripheral nervous system:

In the Nitrosourea studies, Koestner states, most of the longer living animals developed neurinomas. However, no tumors of the peripheral nervous system were seen in the 2,4-D study.

10. Tumor induction outside the nervous system:

Most systemic carcinogens produce extraneural tumors in addition to neurogenic tumors. These neoplasms include leukemias, lymphomas, carcinomas of various organs and sarcomas. However, no increased incidence of extraneural tumors was seen in the 2,4-D study.

11. Genotoxicity, mutagenicity, chromosomal aberrations:

2,4-D was negative when tested in the Ames test, the erythrocyte micronucleus test in mice, the dominant lethal in mice and in human lymphocytes, according to Koestner's assessment. 2,4-D only tested positive in Saccharomyces cerevisiae and gave mixed results in tests with fruit flies (Drosophila melanogaster). Koestner claims that overall, these results indicate little or no mutagenic potential for man.

Dr. Koestner claims that this clustering of gliomas in the high dose male group is most likely due to chance, and statistical analyses of these numbers are only meaningful when some or all of the above criteria are met. In this circumstance, the increased incidence of tumors in the high dose group is just another example of biological variation.

13544

R054904

Chemical:	**2-4,D**
PC Code:	**030001**
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/22/86 12:00:00 AM**
File ID:	**00000000**
Accession Number:	**412-04-0043**

HED Records Reference Center
12/03/2003